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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SMK/LP6150866	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/02645	International filing date *(day/month/year)* 17.06.2003	Priority date *(day/month/year)* 21.06.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/10		
Applicant PLANT BIOSCIENCE LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.01.2004	13.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Chakravarty, A Telephone No. +49 89 2399-8536

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/02645

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-39 as originally filed

Claims, Numbers

1-35 as originally filed

Drawings, Sheets

1/9-9/9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-7,14-25,27,28,30,31,-35
	No:	Claims	1-3,8-13,26,29
Inventive step (IS)	Yes:	Claims	31-35
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-35
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The application concerns the cloning of the hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (HQT) from tobacco leaves. Two clones were found (SEQ IDs 3 and 4) which encode two proteins (SEQ IDs 1 and 2) which have 90% identity with each other and (pg. 5) have 81-83% identity to the known N-hydroxycinnamoyl / benzoyltransferase nucleic acid sequence (AB035183) from sweet potato.

2. Reference is made to the following documents:

D1: DATABASE EMBL [Online] EBI; 27 November 1999 (1999-11-27) KIKUCHI Y. ET AL.: "Ipomoea batatas hcbt mRNA for N-hydroxycinnamoyl / benzoyltransferase, complete CDs." Database accession no. AB035183 cited in the application

D2: DATABASE SWISSPROT [Online] 1 May 2000 (2000-05-01) "Ipomoea batatas N-hydroxycinnamoyl/benzoyltransferase" Database accession no. Q9SST8 cited in the application

D3: LOTFY SAMIA ET AL: "Hydroxycinnamoyl-CoA: Transferases in higher plants. II. Characterization in Cichorium endivia and Raphanus sativus and comparison with other plants." PLANT PHYSIOLOGY AND BIOCHEMISTRY (MONTROUGE), vol. 32, no. 3, 1994, pages 355-363, ISSN: 0981-9428 cited in the application

D5: LOTFY S ET AL: "PARTIAL PURIFICATION AND CHARACTERIZATION OF HYDROXYCINNAMOYL COA TRANSFERASES FROM APPLE AND DATE FRUITS" PHYTOCHEMISTRY (OXFORD), vol. 31, no. 3, 1992, pages 767-772, ISSN: 0031-9422 cited in the application

D6: RHODES M J C ET AL: "PURIFICATION AND PROPERTIES OF HYDROXY CINNAMOYL COENZYME A QUINATE HYDROXY CINNAMOYL TRANSFERASE FROM POTATOES" PHYTOCHEMISTRY (OXFORD), vol. 18, no. 7, 1979, pages 1125-1130, ISSN: 0031-9422 cited in the application

D7: ULBRICH B ET AL: "PARTIAL PURIFICATION AND PROPERTIES OF

HYDROXY CINNAMOYL COENZYME A QUINATE HYDROXY CINNAMOYL TRANSFERASE FROM HIGHER PLANTS" PHYTOCHEMISTRY (OXFORD), vol. 18, no. 6, 1979, pages 929-934, EN ISSN: 0031-9422 cited in the application

Novelty

3. Claims 1-3 are defined only in functional terms without specifying a particular nucleic acid or amino acid sequence. These claims lack novelty over nucleic acid sequence AB035183. This also applies to dependent claims 8-13.

3.1 Applicant argues that the D1 sequence is merely a database entry; there is no clear and unambiguous disclosure in D1 of an isolated nucleic acid sequence encoding an HQT. The D1 sequence would not actually have been expressed. Claim 1 relates to an isolated nucleic acid sequence encoding an HQT.

3.2 The IPEA does not agree. The claim is directed to any nucleic acid sequence encoding a protein having HQT activity, regardless of the extent of this activity. Since claims are not limited by any particular sequence, D1 is regarded as novelty destroying.

3.3 Applicant argues that Claim 11 is independently novel over D1 because this claim refers to the complement of the HQT sequence. D1 does not disclose the complement of an HQT sequence.

3.4 The IPEA dos not agree. The disclosure of one DNA strand implicity discloses also the complementary strand. Claims 14-25 are novel over D1 as it makes no mention of any of the subject-matter claimed in said claims. However, the subject-matter of these claims lacks inventive step (see below).

3.6 Claims 26 and 29 lack novelty over the polypeptide of D1.

3.7 Applicant argues that Claim 26 relates to an isolated polypeptide having HQT activity. D1 provides a "translation" sequence as predicted from the associated nucleic acid sequence. The sequence is annotated as having a different enzyme activity, not having HQT activity. There is thus no disclosure in D1 of a polypeptide having HQT activity. Furthermore, D1 merely provides a predicted translation product: the actual polypeptide would not have been expressed.

3.8 The IPEA does not agree. As set out above, the lack of specification of any particular sequence or any level of HQT activity means that the putative protein sequence of D1 is considered as falling within the scope of claim 26.

3.9 Claims 27 and 28 are novel over the prior art. The further purification of the

particular claimed polypeptides from tomato and tobacco vis-a-vis D3-D7 enables their sequencing and is therefore regarded as a "new element".

Inventive step

4. Claims 1-13 lack inventive step. It is considered that it would have been obvious to prepare the presently claimed nucleic acids and proteins, bearing in mind that the protein was known in the art from both D1 and D3-D7.

4.1 Applicant argues that there was doubt in the art that the enzymes partially purified in the cited papers by Lofty, Rhodes and Ulbrich were actually the HQT enzymes responsible for chlorogenic acid production in plants. Later publications in the prior art disclose that chlorogenic acid is produced by a different route, and thus teach away from HQT enzymes. Moreover, preparation of HQT nucleic acids and proteins involved inventive activity due to a lack of reasonable expectation of being able to succeed starting from the teachings of the prior art. Although HQT enzyme activity had been shown in the cited papers by Lofty, Rhodes and Ulrich, preparation of the claimed nucleic acids and proteins required the application of non-obvious techniques and modifications to the prior art purification procedure. The applicant only succeeded in cloning the claimed nucleic acid sequences after considering various approached and after several attempts. The inventors had to develop additional purification steps and devise a new purification protocol, including multiple column purifications which was not routine. The purification proved to be extremely difficult and took about 8 months. Even after optimising the purification method, the inventors had to repeat the purification procedure eight times over, as the first seven runs were unsuccessful. Only after the eighth repeat of the procedure did the inventors obtain a product that could be used for analysis. A particular problem was the number of false positive results obtained. The protein fractions showing HQT activity generally still contained significant levels of other proteins, making it virtually impossible to identify which protein was responsible for the HQT activity. Luckily, on the eighth repeat of the method, the SDS-PAGE gel showed a protein band whose size corresponded to proteins of the acyltransferase family.
The applicants are of the view that the purification and cloning procedure was highly inventive and would not have been attempted by the ordinary skilled person with any expectation of success.

4.2 The IPEA does not agree. The three pathways cited were presented in the art as alternatives, as can be taken from Niggeweg et al (D8). The IPEA is of the opinion that this would not have led the skilled person to question the validity of any of the pathways, rather they would have be been viewed as alternatives. Moreover, this

may be irrelevant to assessment of inventive step, as starting from D3-D7, the problem to be solved could be seen as provision of the nucleic acid sequence encoding the known HQT proteins. The IPEA remains of the opinion that this problem could have been solved in an obvious manner.
The purification process, while certainly involving real difficulties, is nevertheless considered as being within the ability of the skilled person.
Also the process does not necessarily confer an inventive step on the products. In the present case an alternative approach could also have led to the claimed sequences without need of inventive step. Starting from D1, the skilled person faced with the problem of finding related sequences in other plants would, due to the high homology would have come to the claimed molecules.

4.3 Claims 12 and 13 lack inventive step over AB035183 which can be used to design the claimed primers without inventive effort. The claim as it stands is not limited to primers specifically useful for detecting SEQ ID 3 or 4.

4.4 On the other hand the IPEA accepts inventive step for the subject-matter of claims 31-35 for the reasons set out in the applicant's reply.

4.5 Recognition of inventive step for remaining the claims is dependent on such a step being demonstrated for the nucleic acid claims.